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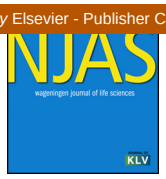

Nutritional qualities and antioxidant activity of three edible oyster mushrooms (*Pleurotus* spp.)

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ABSTRACT

Three species of oyster mushrooms (*Pleurotus* spp.) that are cultivated mostly throughout the year in the plains of India were studied for their nutritional value and their antioxidant properties. Highest protein content was found in *Pleurotus florida* (22–25% dw) followed by *Pleurotus citrinopileatus* (20–22% dw) and *Pleurotus pulmonarius* (15–18% dw). Cholesterol content was in the range of 0.6–0.8% dw, making them low-cholesterol, proteinaceous food. The antioxidant properties of the three species were of both enzymatical and non-enzymatical nature. Reducing power, chelating activity on Fe^{2+} and total phenol contents were higher in *P. florida* than in *P. pulmonarius* and *P. citrinopileatus*. With regard to antioxidative enzymes, *P. florida* had the highest peroxidase and superoxide dismutase activity whereas maximum catalase activity was found in *P. pulmonarius*. *P. florida* had higher antioxidative activity than *P. pulmonarius* and *P. citrinopileatus* thereby highlighting its nutraceutical values along with nutritional qualities.

1. Introduction

Oxidation is essential for all living organisms for the production of energy to fuel their biological processes. However, oxygen-centred free radicals and other reactive oxygen species that are continuously being produced *in vivo* may result in cell death and tissue damage. Oxidative damage caused by these free radicals may be related to ageing and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis [1]. Almost all organisms are well equipped with several defence systems against free-radical damage by oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or by chemical compounds such as α-tocopherol, ascorbic acid, carotenoids, polyphenolic compounds and glutathione [2,3]. These systems are insufficient to prevent damage entirely [4]. However, antioxidant supplements or antioxidant-containing foods are important in the human diet to prevent or to reduce oxidative damage [5,6]. The restriction in the use of synthetic antioxidants, such as BHA (2-tert-butyl-4-methoxyphenol) and BHT (2,6-ditert-butyl-4-methylphenol), has led to an increased interest in natural antioxidant substances [7,8]. Natural antioxidants are being extensively studied for their properties to protect organisms and cells from damage brought about by oxidative stress, the latter being considered a cause of ageing and degenerative diseases. The antioxidants present in foods, especially vegetables, are phenolic compounds (phenolic acids and flavonoids), carotenoids, tocopherol and ascorbic acid [5,9], which are important protective agents for human health [10,11].

Mushrooms are rich sources of those compounds [12–15] and their activities are determined by spectrophotometric techniques [16–18]. Progressively, electrochemical techniques have been developed and tested as an alternative tool for the evaluation of different food extracts, expressed in terms of 'antioxidant power', due to their quickness, simplicity and low cost [19–23].

Substances that may be considered a food or part of a food and that provide medical or health benefits like the prevention and treatment of diseases are referred to as nutraceuticals [24,25]. Mushrooms have become attractive as a functional food and as a source for the development of drugs and nutraceuticals [5,18,26] due to their antioxidant [12,27], antitumor [28] and antimicrobial properties [13,29]. Besides their pharmacological features [30], mushrooms are becoming more important in our diet thanks to their nutritional value, which is related to high protein and low fat/energy contents [31,32]. Mushrooms accumulate a variety of secondary metabolites, including phenolic compound, polyketides,

Abbreviations: SOD, superoxide dismutase; CAT, catalase.

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terpenes, variegatic acid, diboviquinone and steroids [33,34]. Most of their antioxidative potential is the result of the redox properties of phenolic compounds that allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [35].

The study included three species of *Pleurotus: P. florida* (Mont.) Singer, *P. pulmonarius* (Fr.) Quel. and *P. citrinopileatus* Singer. These mushrooms, which are cultivated mostly throughout the year in the plains of India, were studied for their nutritional value (protein and cholesterol content) and their enzymatic and non-enzymatic antioxidative properties in order to establish their potential for human consumption and health.

2. Materials and methods

2.1. Mushrooms

Strains of *Pleurotus florida* (Mont.) Singer (Khatun, 02-11), *Pleurotus pulmonarius* (Fr.) Quel. (Khatun, 03-11) and *Pleurotus citrinopileatus* Singer (Khatun, 01-11) were obtained from the National Centre for Mushroom Research and Training, Solan, India and were maintained on potato dextrose agar (PDA) slants. Spawn was produced by growing mycelium on wheat grain in autoclavable polypropylene bags (15 cm × 12 cm) following standard methods [36]. Rice straw and water hyacinth were used as substrates for growing mushrooms. The aerial parts (stems and leaves only) were sun dried; the roots were discarded as these were reported to absorb heavy metals [37]. Both substrates were chopped into small pieces (3–5 cm) and steeped overnight in tap water. After draining the excess water the substrates were pasteurized at 80 °C for 90 min. After cooling down, 2 kg of moistened substrate was transferred to transparent polythene bags, 50 cm × 35 cm, perforated with 20–25 evenly distributed holes. The substrates were used either alone (rice straw or water hyacinth) or in combination (rice straw + water hyacinth). The bags were then inoculated with wheat spawns at 5% (w/w) on a wet weight basis following multilayer technique and incubated in the semi-dark at temperatures conditioned between 26 °C and 28 °C during winter (December to February) and pre-summer (February to April) seasons. After a spawn run period of 13–19 days (according to substrate combination), the polythene bags were removed. The temperature and relative humidity (RH) inside the cropping room were then maintained at 18–22 °C/24–28 °C and 75–80%/80–85%, respectively, during fruit body formation.

Fresh fruiting bodies of the three *Pleurotus* species, cultivated at the Mycology and Plant Pathology Laboratory, Department of Botany, Burdwan University, were cleaned and washed with distilled water. They were cut into small pieces, dried to a constant weight in a hot-air oven at 60 °C (moisture content for *P. florida* 82.67 ± 8.1% fw, for *P. pulmonarius* 80.57 ± 9.5% fw and for *P. citrinopileatus* 79.27 ± 11.7% fw), ground to a particle size of 40 mesh by using mortar and pestle and stored at −4 °C in airtight containers until further use.

2.2. Protein

Protein content of the mushrooms was measured according to Bradford [38]. Dry (20 mg) tissue was crushed with 2.5 mL methanol and a pinch of neutral sand and centrifuged at 5000 rpm for 15 min. The pellet was taken discarding the supernatant. The pellet added with 2.5 mL of 1 M NaOH was kept at 80 °C for 1 h and cooled at room temperature. The volume was adjusted to the initial level and centrifuged at 10,000 rpm for 15 min. One tenth of a mL supernatant mixed with 5 mL Bradford reagent [38] was used for measuring protein content. After 5 min, absorbance was recorded at 595 nm in a UV–vis spectrophotometer (Systronics 117) against a control sample.

2.3. Cholesterol

Cholesterol content of the mushrooms was measured following the CHOD/PAP method [39] using a diagnostic kit (Crest Biosystem, Goa, India).

2.4. Catalase

To measure catalase (CAT) content 100 mg of dry mushroom tissue was crushed in a mortar and pestle with 3 mL of phosphate buffer (0.1 M, pH 7.0) at 1–4 °C and centrifuged at 3000 rpm for 15 min. The supernatant was analysed for the enzyme following the method of Luck [40]. The enzyme activity was expressed as units per g dry tissue.

2.5. Peroxidase

Peroxidase activity was estimated following the method of Mahadevan and Sridhar [41] using freshly prepared pyrogallol reagent. The reaction mixture containing 5 mL of freshly prepared pyrogallol reagent (prepared by mixing 10 mL of 0.5 M pyrogallol solution and 12.5 mL of 0.66 M phosphate buffer made up with distilled water to 100 mL) and 1.5 mL of the enzyme extract was placed in a spectrophotometer tube and immediately adjusted to zero absorbance in a spectrophotometer. Half of a mL of 1% H_2O_2 solution was added and the contents were mixed by inverting the tube. The reaction was initiated by the addition of H_2O_2. Enzyme activity was recorded as the change in absorbance per min at 430 nm immediately after the addition of substrate and was expressed as the change in absorbance per min per g dry tissue.

2.6. Superoxide dismutase

Superoxide dismutase (SOD) activity was estimated following the method of Kakkar et al. [42] using Na-pyrophosphate buffer (0.052 M; pH 8.3). The extract was obtained by centrifuging at 10,000 rpm for 15 min. The supernatant was recovered and kept in a tube in an ice bath until further use. Fifty μL enzyme extract, 1.35 mL of double-distilled water, 1.2 mL Na-pyrophosphate buffer, 0.1 mL phenozinemethosulphate and 0.3 mL nitrobluetetrazolium were mixed. Then 0.2 mL NADH was added to initiate the reaction followed by incubation at 39 °C for 90 s. The reaction was terminated by adding 1 mL glacial acetic acid. Four mL n-butanol was added and mixed vigorously in a Vortex mixer and centrifuged at 4000 rpm for 10 min. The upper layer of butanol was taken off and reading was taken at 560 nm against a corresponding blank solution. The assay was based on chromogen production using phenozinemethosulphate and nitrobluetetrazolium in the presence of SOD. One unit of SOD activity is defined as the amount of enzyme that inhibits the rate of reaction by 50% under specified conditions. The enzyme activity was expressed as units per g dry tissue.

2.7. Reducing power and chelating activity on Fe^{2+}

To measure the reducing power and chelating activity on Fe^{2+} 1 g of dry mushroom powder was stirred in 50 mL ethanol and water (3:1, v/v) at 75 °C and centrifuged at 3000 rpm for 1 h. The extract was then filtered using Whatman No. 1 filter paper, the filtrate was collected and dried in a rotary evaporator at 40 °C, transferred into a plastic bottle and stored at −20 °C until further use. The reducing power of the ethanol extract was measured using potassium ferricyanide [43]. The presence of reductants (antioxidants) in the samples would result in reducing Fe^{3+}/ferricyanide complex to the

Table 1
Enzymatic antioxidant properties and total phenol content of *Pleurotus florida*, *Pleurotus pulmonarius* and *Pleurotus citrinopileatus*. Values expressed as means ± standard deviation ($n = 5$).

Pleurotus spp.	Enzymatic antioxidant activity			
	Catalase (CAT, units g^{-1} dw)	Peroxidase ($\Delta A\, g^{-1}\, min^{-1}$)	Superoxide dismutase (SOD, units g^{-1} dw)	Total phenol (μg catechol g^{-1} dw)
P. florida	1116 ± 4	18.76 ± 2.90	347.5 ± 0.06	119 ± 1.6
P. pulmonarius	2264 ± 4	15.12 ± 1.61	260.4 ± 0.08	83 ± 1.6
P. citrinopileatus	1128 ± 2	14.14 ± 1.60	203.7 ± 0.01	64 ± 1.6

ferrous form (Fe^{2+}). The Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm [44]. The reducing power of the ethanolic extract was compared with that of ascorbic acid.

The chelating activity of the ethanolic extracts on Fe^{2+} was measured using the ferrozine reagent [45]. An aliquot of 1 mL of different concentrations (0.1, 0.25, 0.50, 0.75 and 1.00 mg mL^{-1}) of the ethanolic extracts was mixed with 2.5 mL of de-ionized water. The mixture was left to react with $FeCl_2$ (2 mM, 0.1 mL) and ferrozine (5 mM, 0.2 mL) for 10 min at room temperature. The absorbance was measured at 562 nm in a UV–vis spectrophotometer (Systronics 117). A lower absorbance indicates a higher chelating power. Chelating activity on Fe^{2+} of the ethanolic extract was compared with that of ethylenediaminetetraacetic acid (EDTA) and citric acid. Chelating activity was calculated following the equation:

$$\text{Chelating activity}\,(\%) = 1 - \frac{\text{Absorbance of sample at 562 nm}}{\text{Absorbance of control at 562 nm}} \times 100$$

2.8. Total phenol

One hundred mg dry mushroom tissue was placed in 5–10 mL of 80% ethyl alcohol, boiled for 5–10 min in a hot water bath (100 °C) and then cooled in a pan of cold water (20 °C) for 30 min. The tissue was then crushed in a mortar and pestle for 5–10 min and passed through a double-layered cloth. The crushed tissue was again extracted by boiling in 80% alcohol, cooled and then passed through Whatman No. 1 filter paper. Total phenol was estimated using Folin–Ciaocalteu reagent [41]. Catechol was used as the standard. The amount of phenolics was expressed as μg catechol g^{-1} dry tissue.

2.9. Statistical analysis

Data were expressed as means ± standard deviation. Statistically significant differences among the means were determined using one-way ANOVA ($p < 0.01$). Correlations between two antioxidant attributes were determined by Pearson Correlation analysis using Statistical Package for the Social Sciences (SPSS) version 17.0 statistics software.

3. Results and discussion

The results revealed that among the nutritional properties, protein content remained significantly higher ($p < 0.01$) in *P. florida* (23.8 ± 1.30% dw) than in *P. citrinopileatus* (20.8 ± 0.84% dw) and *P. pulmonarius* (16.8 ± 1.30% dw). The insignificant cholesterol contents ($p > 0.05$) of the three mushroom species (*P. florida* 0.68 ± 0.08% dw, *P. pulmonarius* 0.7 ± 0% dw, *P. citrinopileatus* 0.8 ± 0% dw) make them a low cholesterol, protein-rich food. Generally, high protein and low fat levels were also described by previous authors [31,32,46,47]. Oyster mushrooms (*Pleurotus* spp.) are excellently edible and nutritious, and rank among the most widely cultivated mushrooms in the world [48].

As regards the enzymatic antioxidant properties, catalase (CAT) activity (Table 1) was found to be significantly higher ($p < 0.01$) in *P. pulmonarius* than in *P. citrinopileatus* and *P. florida*. Peroxidase and superoxide dismutase (SOD) activities and total phenol content were significantly higher ($p < 0.01$) in *P. florida* than in the two other species. Antioxidants are needed by the human body to combat free radical activities responsible for accelerating ageing processes of tissues and pathologies such as cancer or cardiovascular diseases. Endogenous supply of antioxidants such as CAT, SOD and peroxidase protects the cells against excessive levels of free radicals [49]. The extracts from fruiting bodies of *P. florida* and *P. pulmonarius* occurring in India had significant antioxidant properties [50,51]. Ramesh and Pattar [52] observed that *P. pulmonarius* occurring in Western Ghats of Karnataka, India had significant antioxidant properties and antimicrobial activities. Ethanol extracts from the mycelium of *Pleurotus sajor-caju*, *P. florida* and *P. aureovillosus* have been investigated for their antioxidant capacity, antimicrobial activities and phytochemicals. The levels of phenolic compounds in ethanol extract of *P. sajor-caju*, *P. florida* and *P. aureovillosus* were found to be 6.001 ± 0.04 μg mg^{-1}, 7.501 ± 0.10 μg mg^{-1} and 6.72 ± 0.05 μg mg^{-1}, respectively [53]. The phenolic compounds may contribute directly to antioxidative action [54]. Lee et al. [55] showed that in *P. citrinopileatus*, phenols were the major antioxidant components and their contents were found in the order: fruiting bodies (8.62–12.38 mg g^{-1}) > mycelia grown on fermentation broth (5.84–7.85 mg g^{-1}) > fermentation filtrate (4.80–5.57 mg g^{-1}). The antioxidant properties of compounds are well correlated with the contents of phenolic compounds [56]. Phenols are reported to have good antioxidant [57], antimutagenic and anticancer properties [58]. So, the high total phenol content in *P. florida* in the present study is most likely responsible for its better antioxidant properties.

Our results (Fig. 1) show that the reducing power of the three mushroom extracts increased significantly ($p < 0.01$) with an increase in total phenol content: 0.217, 0.103 and 0.073 A_{700}, at 1.0 mg mL^{-1} in *P. florida*, *P. pulmonarius* and *P. citrinopileatus*, respectively. In addition, this parameter was higher for the three mushrooms extracts than for ascorbic acid. The reducing power

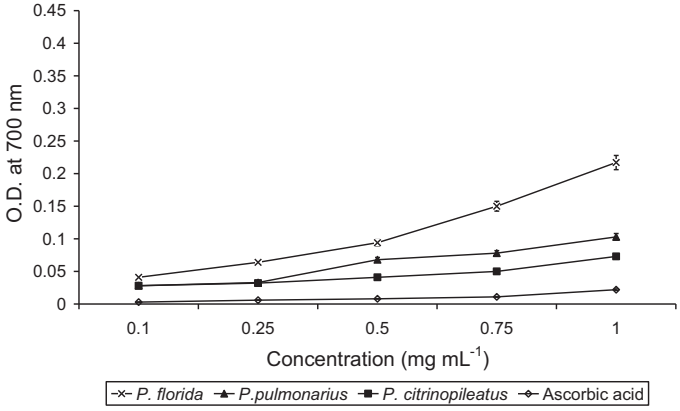

Fig. 1. Reducing power of *Pleurotus florida*, *P. pulmonarius* and *P. citrinopileatus* in comparison with ascorbic acid. Values expressed as means ± standard deviation ($n = 5$).

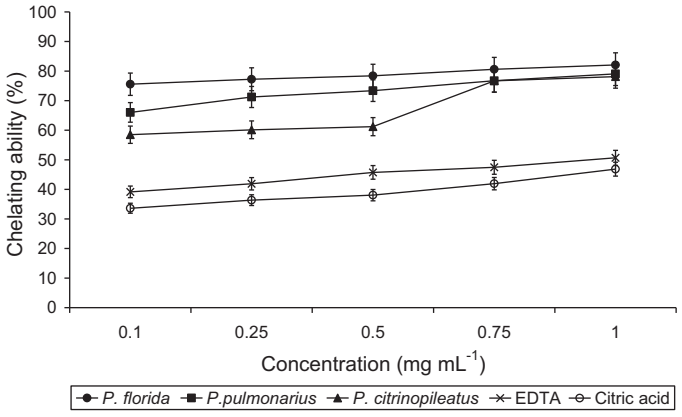

Fig. 2. Chelating ability of *Pleurotus florida*, *P. pulmonarius* and *P. citrinopileatus* in comparison with EDTA and citric acid. Values expressed as means ± standard deviation ($n = 5$).

of the three extracts from fruiting bodies of *P. citrinopileatus* was reported to be 1.03–1.10 at 5 mg mL^{-1}, which was higher than those of mycelia and filtrate [55]. On the other hand, the ethanolic extract of *Ganoderma lucidum* and *Cordyceps sinensis* was reported to have a reducing power of 2.50 and 1.97, respectively, at 1.0 mg mL^{-1} [59]. These values were higher than those obtained in the present study for the three mushrooms. Reductants such as ascorbic acid can react directly with peroxides and also with certain precursors and thereby prevent the formation of peroxide [60]. The reducing power of various extracts might be due to its hydrogen donating ability [60]. So, *P. florida*, *P. pulmonarius* and *P. citrinopileatus* might contain reductants that could react with free radicals to stabilize and terminate free radical chain reactions [61,62].

The mushroom extracts showed a chelating activity on Fe^{2+} that depended on the concentration (Fig. 2). The chelating activity on Fe^{2+} of the *P. florida* extract was higher than that of the *P. pulmonarius* and *P. citrinopileatus* extracts. The chelating activities of the individual mushroom extracts with a concentration of 1.0 mg mL^{-1} (82.07%, 79.04% and 78.14%, respectively) were significantly higher ($p < 0.01$) than that of EDTA (50.66%) and citric acid (46.84%). The chelating abilities of *P. citrinopileatus* in extracts of ethanol, cold water and hot water at 5 mg mL^{-1} were reported to be 46%, 67% and 82%, respectively [55]. The present findings are in line with the results found by Lee et al. [55] in ethanolic extract of *P. citrinopileatus*.

The antioxidant potential of the two commonly used edible mushrooms *P. florida* and *P. eous* was estimated by Imran et al. [63]. *P. florida* had a higher chelating activity against ferrous ions than *P. eous* which corroborates the present findings. Thus the study suggests that the consumption of oyster mushrooms may enhance the immune power of our body against diseases due to free radicals. So they can be used as a dietary supplement along with other foods or as a drug itself.

Chelation property may provide protection against oxidative damage and iron-overload [64]. The chelating ability of a plant extract provides a strategy to avoid free-radical generation and iron-overload through the chelation of metal ion [65]. Since ferrous ions are the most effective pro-oxidants in food systems [66], the high ferrous ion chelating properties of the extracts of *P. florida* would make consumption of this mushroom beneficial.

Chelating ability was positively correlated with CAT for *P. florida* ($r = 0.925$; $p < 0.05$) with total phenol for *P. pulmonarius* ($r = 0.907$, $p < 0.05$) and with peroxidase ($r = 0.879$; $p < 0.05$), CAT ($r = 0.879$; $p < 0.05$) and total phenol ($r = 0.932$; $p < 0.05$) for *P. citrinopileatus*. Peroxidase was also positively correlated with CAT ($r = 1.000$; $p < 0.01$).

The phenolics of the three *Pleurotus* mushrooms showed a high tendency of chelation particularly for iron, leading to the stabilization and termination of radical chain reactions [67–69].

4. Conclusion

Of the three *Pleurotus* spp. studied, *P. florida* has a higher protein content and a much better antioxidative action than *P. pulmonarius* and *P. citrinopileatus*; highlighting its nutraceutical value. The *Pleurotus* mushrooms are comparable to the best antioxidants, which is attributed to their catalase, phenolics and peroxidase contents.

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